PHARMACOGENETICS IN PERSPECTIVE

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This conference was conceived at a meeting last year in Philadelphia when Dr. Bob Smith, Dr. Wendell Weber, and I saw each other and came to talk. A starting point of our discussion was my interest in the work of Dr. Emil Skamene and his colleagues in Montreal. They had found and reported a human gene that causes resistance to tuberculosis, a resistance that plays a role before the immune system comes in.

The purpose of this conference is to put pharmacogenetics, ecogenetics, and inborn resistance to infectious diseases into a broad perspective. They all deal with genetic factors that shape our response to one or another selected environmental force. I say selected force, because there are others that we will not consider here. For instance, there are big differences between people’s ability to adjust to living at high or low temperature. There are differences in the ability to live at high altitudes. Nevertheless, by using this meeting to look together at drugs, foods, and infectious diseases, we are broad-minded but we are not comprehensive. I always tended to look narrowly upon drugs and toxicants, as if they were unique potential disturbers of life. I hope that this meeting will stimulate broader outlooks in all of us.

Let me talk about pharmacogenetics; it is the professional specialty that I grew up with (Kalow, 1962). It started with great interest in the genetic variability of all sorts of drug-metabolizing enzymes, be it cholinesterase, paraoxonase, glucose-6-phosphate dehydrogenase, one of the many transferases, P450 cytochromes like CYP2D6, CYP2C19, and other catalysts (Kalow, 1992; Price-Evans, 1993; Weber, 1997). More recently came the focus on drug receptors, like dopamine, serotonin, or [i]N-methyl-aspartate receptors (Propping and Nothen, 1995; Seeeman et al., 1996; Weber, 1997). Currently, an interest starts in pharmacogenetics of transporters, such as P-glycoproteins (Kim et al., 1998; Fromm et al., 1999). These interests in single gene products are understandable because they are often clinically important. Nevertheless, this interest in monogenic factors is still peculiar, since most differences between people are multifactorial.

Metabolic differences with pharmacokinetic consequences may be caused by numerous factors. There may be differences in production or stability of RNA, by differences of transcription or translation, by differences of enzyme formation or degradation, by improper protein folding, by variable concentrations of inducers or inhibitors, which may be exogenous chemicals like drugs or food products, or endogenous ones like hormones. Pharmacokinetic differences may be caused by drug interactions, not to speak of such factors like age, gender, or disease-controlled gene interactions. In addition there are physiological factors like epigenetic gene inactivation (Laird and Jaenisch, 1996; Gasser et al., 1998) or genomic imprinting (Skuse, 1999). All or many of these and additional factors may work together to cause the ever present person-to-person differences in drug response. In pharmacology, the existence of such ever present differences is traditionally defined by the term “ED50”, indicating the dose of a drug that will affect 50% of any population under study (Trevan, 1927). Thus, such differences have long been recognized but have never been a major topic of pharmacogenetics.

I will emphasize in my talk multifactorial differences between people in their response to drugs. Let me mention two projects pertaining to this topic.

Multifactorial Differences and the Edge Effect

Statistically, multifactorial differences are characterized by Gauss distributions, that is by “normal” distribution curves. This illustration gives phenylbutazone as an example. In addition, the human capability to metabolize caffeine is usually normally distributed (Kalow and Tang, 1991; Tang et al., 1994). A conclusion that can be wrong in pharmacology is too much emphasis on the mean value associated with a normal distribution. It can lead to wrong emphasis if one compares different mean values in different populations (Kalow, 1992; Kalow and Bertilsson, 1994). Let us take an example (Fig. 1). Let us assume we measure the elimination rate of a drug in two populations, and we find that the means differ by one standard deviation. From a clinical point of view, this difference may be insignificant because of the spread of the data in each population; most of the distribution curves are overlapping. However, let us assume that the lowest 2% of population A (the population with the higher mean value) runs into trouble because of slow drug elimination. It would mean that about 16% of the other population might run into the same trouble, an 8-fold difference. The size of the differences would increase if we move toward the outer edge of the distribution curves. I like to call this the edge effect (Kalow, 1992; Kalow and Bertilsson, 1994).

My colleague, Dr. Ozdemir, recently pointed out that this edge effect will be important in many studies involving pharmacological comparisons of average effects. An example is bioequivalent studies, that is, studies conducted to compare an original drug with a substitute. Another potentially important case is studies of drug interaction, when one drug inhibits the metabolism of another. It may not be appropriate to consider just the mean value of the elevation of the drug level caused by the interfering drug.

Repeated Drug Administrations: Replacement of Twin Studies

Dealing with multifactorial controls of drug response leads to another problem. Multifactorial usually means that both genetic and environmental factors contribute to variation. The questions, how much of a given variation is genetically controlled, and how much is environmentally controlled then often arises. The general way to determine heritability is to use twin studies, that is, to compare the differences between the members of identical and fraternal twin pairs (Vesell, 1992). It is often difficult or expensive to recruit twins. The
use of twins is still a necessity if one tries to assess the heritability of stable factors like size or intelligence. Since drug effects come and go, we can, in pharmacology, achieve the same result as with twin studies by testing the metabolism or the effect of a drug when given twice or more often to a group of people (Kalow et al., 1998, 1999a,b).

Assuming we have twenty subjects, and we give each person a drug on several occasions, spaced so that the previous drug effect has worn off, we then measure the differences between people in terms of the standard deviation (SD), and we measure the within-subject variability of the results of repeated drug administrations in terms of SD_w. We then square the standard deviation values and use the equation \( r_{GC} = (SD_w^2 - SD_a^2)/SD_b^2 \) as an indication of the genetic contribution to the measured pharmacological variation. Various criteria can be used to judge data quality to avoid major mistakes. Furthermore, we can use an F-test to determine the confidence limits of our measurement (W. Kalow, submitted). This method has given us some very interesting results. Ozdemir et al. (2000) analyzed data by Kashuba et al. (1998), who used intravenous injections of midazolam to test the activity of hepatic CYP3A4; analysis of the data by the above equation showed the genetic contribution to account for 96% of the enzyme activity, with 95% confidence limits of 92 and 98%. This is remarkable, since many tests for allelic variation of the CYP3A4 gene have been unsuccessful. It means that an unknown genetic factor must control enzyme formation.

Another interesting observation of Ozdemir et al. (2000) came from analyzing data by Ohlman et al. (1993), who investigated the pharmacokinetics of cyclosporine during day and night. The genetic contribution was much higher at night than during the day; food consumption, liver blood flow, and other factors are clearly less important. Fromm MF, Kim RB, Stein CM, Wilkinson GR and Roden DM (1999) Inhibition of P-glycoprotein-mediated drug transport: A unifying mechanism to explain the interaction between digoxin and quinidine. Circulation 2:552–557.


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References


